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INCREASED BREAST CANCER RISK AFTER A MULTIPLE BIRTH IN IVF-TREATED WOMEN: A NATIONWIDE DUTCH COHORT STUDY

SUBMITTED

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ABSTRACT

Background

Breast cancer risk is temporarily increased after a full-term pregnancy and declines thereafter, possibly due to increased levels of gonadal and placental hormones during pregnancy. Inconsistent results, however, have been reported after twin pregnancies with higher hormone levels. Among women treated with in-vitro fertilization (IVF), for whom the number of embryos available for implantation is known, we recently observed that a multiple birth after implantation of all transferred embryos is associated with higher levels of vascular endothelial growth factor (VEGF). As VEGF is involved in breast cancer progression, we studied the effects of embryo implantation and a multiple birth on breast cancer risk in a nationwide Dutch cohort of IVF-treated women.

Methods

We performed a cohort analysis among 12,589 women who had been treated with IVF between 1983-1995 and completed a risk factor questionnaire between 1997-1999. Data on IVF treatment were obtained from medical records. Breast cancer cases were ascertained through linkage with the population-based Netherlands Cancer Registry. Breast cancer risks by parity and having singletons or multiples were estimated with Cox regression.

Findings

There were 1,688 women (13·4%) with multiples, 6,027 (47·9%) with singletons, and 4,874 (38·7%) nulliparous women. Breast cancer occurred in 317 women of whom 57 had multiples. Breast cancer risk was 1·44 times higher in mothers of multiples than in mothers of singletons (95% CI (1·06-1·97)). Risk was highest in women who gave birth to multiples from all embryos transferred (adjusted HR 1·86, 95% CI (1·01-3·43)), and lower for those with multiples after incomplete implantation (adjusted HR 1·31, 95% CI (0·76-2·25)).

Interpretation

A woman's potential to implant all embryos transferred may be associated with breast cancer risk. Further research is needed to confirm our results and to identify the underlying biological mechanisms.

INTRODUCTION

Breast cancer is the most frequently diagnosed cancer and leading cause of cancer death among women worldwide [1]. Epidemiologic and experimental studies suggest that the hormones oestrogen and progesterone are strongly involved in breast carcinogenesis [2]. These hormones stimulate cellular proliferation in the breast, thereby increasing the chance for accumulation of somatic mutations during cell division [3]. Many risk factors for breast cancer, including several reproductive factors (age at menarche and menopause, parity and age at first birth) are believed to exert their effects by changing a woman's lifetime exposure to oestrogens and progesterone [2]. After a full-term pregnancy, there is a temporary increase in breast cancer risk followed by a long-lasting decrease in risk. This has been attributed to increased levels of gonadal and placental hormones during pregnancy. As concentrations of oestrogen and progesterone are higher in women with a multiple pregnancy than in women with a singleton pregnancy [4-6], women with a multiple pregnancy might have a higher risk of breast cancer. On the other hand, a multiple pregnancy is also associated with higher levels of alpha-fetoprotein and sex-hormone binding globulin, which both contain anti-oestrogenic properties. It has therefore been argued that women with a multiple pregnancy might as well have a decreased breast cancer risk [7-11].

To date, several studies have reported on breast cancer risk after a multiple pregnancy in the general population [12]. These studies have shown inconsistent results and a recent meta-analysis of 17 studies did not reveal a significant association between a multiple pregnancy and breast cancer risk [12]. In contrast to a natural multiple pregnancy, for most pregnancies after in-vitro fertilization (IVF) the number of embryos available for implantation is known. We recently observed that women who gave birth to multiples from all embryos transferred had higher levels of vascular endothelial growth factor (VEGF) prior to any treatment [13], an identified soluble endometrial factor that plays an important role during human embryonic implantation [14]. As VEGF is also involved in breast cancer progression, possibly by promoting neovascularisation [15-18], we wondered whether the potential to successfully implant all embryos after multiple embryo transfer could be associated with breast cancer risk.

This question and the inconsistent results on breast cancer risk after a multiple birth prompted us to examine the effects of embryo implantation and a multiple birth in a nationwide Dutch cohort of IVF-treated women.

METHODS

Study population

In 1995–1996, we identified a nationwide historical cohort of 19,861 subfertile women who received at least one IVF cycle with ovarian stimulation between 1983 and 1995 in one of the twelve IVF centres in the Netherlands (OMEGA-study). This study was originally designed to examine the long-term effects of ovarian stimulation in IVF-treated women on the risk of hormone-related cancers. A more detailed description of the study population and study procedures has been published previously [19-20].

Data collection

In brief, between 1997 and 1999, 19,275 IVF-treated women were sent a questionnaire, a study information letter, and a brochure. Each participant was asked written informed consent for medical record data abstraction and future linkage with disease registries. The questionnaire ascertained information on women's date of birth, weight, height, reproductive histories (number of pregnancies, pregnancy outcome (singleton or multiple, gestational age, date of delivery), and child characteristics), subfertility treatment, use of exogenous hormones, lifestyle factors, and (family) history of cancer. Data from medical records were collected by trained abstractors. For each IVF cycle, we recorded cause of subfertility, date, number of oocytes collected, number of embryos transferred, and pregnancy outcome. Due to limited funding we could only complete medical records abstraction for 9 out of 12 centres (i.e. 76% of women).

Breast cancer incidence

Cancer incidence from January 1989 to July 2009 was ascertained through linkage with the population-based Netherlands Cancer Registry (NCR) by using an earlier developed record linkage protocol [19]. Because the NCR did not fully cover the Netherlands before January 1989, cases before this date were only identified by self-report in the questionnaire. For each breast cancer case, we received information on date of diagnosis and morphology. Ductal carcinomas in situ were not included as breast cancer cases. Vital status as of June 2009 was obtained by linkage with the Central Bureau for Genealogy, which keeps computerized records of all deceased persons in the Netherlands since 1994.

Statistical analysis

The study population consisted of all women who completed the questionnaire (n=13,715, response rate 71%). Women diagnosed with cancer before entering the cohort or before their first IVF treatment (n=43) and those who explicitly refused future linkage (n=831) were excluded. For 252 women reproductive data were missing, leaving 12,589 women in the analytic cohort. Pregnancies with a gestational age under 24 weeks were ignored as literature has shown no association

between miscarriages and breast cancer risk [21]. We calculated the cumulative incidence of breast cancer and compared breast cancer risks between mothers of singletons (reference), mothers of multiples, and nulliparous women using Cox regression models. Follow-up started at first IVF treatment and ended at breast cancer diagnosis, death, emigration, or final date of NCR follow-up (July 1st 2009), whichever came first. Breast cancer hazard ratios (HR) and 95% confidence intervals (95% CI) were calculated with age as the time scale and ever having given birth to a singleton or multiple as a time-dependent variable. Women with a singleton birth before a multiple birth contributed person-time to the nulliparous category until the singleton birth, to the singleton category until the multiple birth and to the multiple category thereafter. No evidence of non-proportional hazards was observed when including interactions (of relevant variables) with log-transformed attained age. P-values for trend were calculated according to standard methods [22]. Although no confounders were identified during the forward stepwise confounder selection (based on a 10% change), we decided to also present HRs adjusted for relevant potential confounders described in literature, i.e. year of first IVF treatment, number of IVF cycles, height, and age at first birth. It was not possible to adjust for both age at first birth and parity due to the presence of collinearity. Analyses were performed with the statistical software package SAS (SAS software version 9·2; SAS Institute Inc., Cary, NC, USA).

There were 54 breast cancers diagnosed between first IVF treatment and questionnaire completion, and the above mentioned analyses therefore assume that questionnaire response of breast cancer cases compared to non-cases is non-differential with respect to birth history. To exclude this potential selection bias, we performed sensitivity analyses in which the follow-up time started at questionnaire completion. End of follow-up was defined as described previously. Since no data on singleton or multiple births were available after questionnaire completion, covariates in these analyses were not time-dependent. HRs were adjusted for age at questionnaire completion, number of IVF cycles, maternal height, and age at first birth. Analyses were performed using SPSS statistical software (SPSS for Windows, Rel 17·0. Chicago: SPSS Inc.). A p-value of <0·05 was considered significant. All tests of statistical significance were two sided. Missing values for confounder variables were imputed by the median (TABLE I).

To examine the association between multiple birth after multiple embryo transfer and breast cancer risk, subgroup-analyses were performed including only pregnancies conceived after IVF treatment. For each first singleton or multiple pregnancy, we compared the number of children delivered with the number of transferred embryos. Subsequently, births were categorised into three groups: singleton births, multiple births after implantation of all embryos transferred, and multiple births after implantation of part of the embryos transferred.

RESULTS

Population characteristics

TABLE I shows the patient and treatment characteristics of the study population. At questionnaire completion, 1,688 women (13·4%) had given birth to multiples, 6,027 (47·9%) had given birth to singletons only, and 4,874 (38·7%) remained nulliparous. Of the mothers of multiples, 1,455 women (86·2%) delivered twins, 208 (12·3%) delivered triplets, and five (0·3%) delivered quadruplets. Fourteen women (0·8%) delivered two twins and six women (0·4%) delivered both twins and triplets. Of the mothers of multiples, 693 women (41·0%) also gave birth to a singleton, of whom 174 women (25·1%) had the multiple birth as their first birth. Mothers of multiples were younger at first IVF treatment and received fewer IVF cycles than nulliparous women and mothers of singletons. Follow-up time since first IVF treatment did not differ between nulliparous women and women with a singleton or multiple birth (median 16·7 years).

TABLE I **Characteristics of the IVF study population**

	Total study population	Nulliparous	Singleton births	Multiple births [A]
No. of participants (%)	12,589	4,874 (38.7)	6,027 (47.9)	1,688 (13.4)
Year of birth (%)				
⩽ 1953	1,528 (12.1)	731 (15.0)	705 (11.7)	92 (5.5)
1954-1957	3,215 (25.5)	1,305 (26.8)	1,556 (25.8)	354 (21.0)
1958-1961	4,122 (32.7)	1,461 (30.0)	2,013 (33.4)	648 (38.4)
⩾ 1962	3,724 (29.6)	1,377 (28.3)	1,753 (29.1)	594 (35.2)
Age at questionnaire completion (%)				
< 35	2,768 (22.0)	1,049 (21.5)	1,291 (21.4)	428 (25.4)
35-39	4,983 (39.6)	1,769 (36.3)	2,420 (40.2)	794 (47.0)
⩾ 40	4,838 (38.4)	2,056 (42.2)	2,316 (38.4)	466 (27.6)
Age at first IVF treatment (%)				
< 30	3,011 (23.9)	1,100 (22.6)	1,397 (23.2)	514 (30.5)
30-34	5,713 (45.4)	2,086 (42.8)	2,768 (45.9)	859 (50.9)
⩾ 35	3,865 (30.7)	1,688 (34.6)	1,862 (30.9)	315 (18.7)
Mean (SD)	32.9 (4.1)	33.3 (4.3)	33.0 (4.0)	31.8 (3.5)
Calendar year of first IVF treatment (%)				
⩽ 1986	276 (2.2)	118 (2.4)	127 (2.1)	31 (1.8)
1987-1989	1,426 (11.3)	580 (11.9)	644 (10.7)	202 (12.0)
1990-1992	5,328 (42.3)	1,951 (40.0)	2,599 (43.1)	778 (46.1)
1993-1995	5,301 (42.1)	2,082 (42.7)	2,559 (42.5)	660 (39.1)
⩾ 1996	258 (2.0)	143 (2.9)	98 (1.6)	17 (1.0)
Subfertility diagnosis (%)				
Male	3,418 (27.2)	1,365 (28.0)	1,560 (25.9)	493 (29.2)
Tubal	4,094 (32.5)	1,541 (31.6)	1,999 (33.2)	554 (32.8)
Unexplained	2,808 (22.3)	1,065 (21.9)	1,381 (22.9)	362 (21.4)
Mix / other	2,071 (16.5)	833 (17.1)	994 (16.5)	244 (14.5)
Unknown [B]	198 (1.6)	70 (1.4)	93 (1.5)	35 (2.1)
Number of IVF cycles (%)				
1 – 2 cycles	4,823 (38.3)	1,307 (26.8)	2,585 (42.9)	931 (55.2)
3 – 4 cycles	5,065 (40.2)	2,375 (48.7)	2,197 (36.5)	493 (29.2)
⩾ 5 cycles	2,629 (20.9)	1,160 (23.8)	1,210 (20.1)	259 (15.3)
Unknown [B]	72 (0.6)	32 (0.7)	35 (0.6)	5 (0.3)

Age at first child (%)	Total study population	Nulliparous	Singleton births	Multiple births [A]
< 25	1,004 (13.0)	NA	863 (14.3)	141 (8.4)
25-30	1,652 (21.4)	NA	1,281 (21.3)	371 (22.0)
30-34	3,002 (38.9)	NA	2,199 (36.5)	803 (47.6)
⩾ 35	1,962 (25.4)	NA	1,597 (26.5)	365 (21.6)
Unknown [B,C]	95 (1.2)	NA	87 (1.4)	8 (0.5)
Parity at questionnaire completion (%)				
0	4,874 (38.7)	4,874 (100.0)	NA	NA
1	4,552 (36.2)	NA	3,571 (59.3)	981 (58.1)
2	2,543 (20.2)	NA	1,958 (32.5)	585 (34.7)
⩾ 3	620 (4.9)	NA	498 (8.3)	122 (7.2)
Multiple birth (%)				
Twins	1,455 (86.2)	NA	NA	1,455 (86.2)
Triplets	208 (12.3)	NA	NA	208 (12.3)
Quadruplets	5 (0.3)	NA	NA	5 (0.3)
Two multiple births	20 (1.2)	NA	NA	20 (1.2)
Height (%)				
< 164 cm	2,753 (21.9)	1,161 (23.8)	1,295 (21.5)	297 (17.6)
164-170	4.299 (34.1)	1,650 (33.9)	2,074 (34.4)	575 (34.1)
170-173	2,623 (20.8)	960 (19.7)	1,292 (21.4)	371 (22.0)
⩾ 174	2,770 (22.0)	1,037 (21.3)	1,304 (21.6)	429 (25.4)
Unknown [B]	144 (1.1)	66 (1.4)	62 (1.0)	16 (0.9)
Years of follow-up since first IVF treatment (%)				
< 10	345 (2.7)	163 (3.3)	137 (2.3)	45 (2.7)
10-14	2,425 (19.3)	1,056 (21.7)	1,106 (18.4)	263 (15.6)
15-19	8,531 (67.8)	3,110 (63.8)	4,204 (69.8)	1,217 (72.1)
⩾ 20	545 (11.2)	580 (9.6)	163 (9.7)	1,288 (10.2)
Mean (±SD)	16.7 (3.0)	16.6 (3.2)	16.7 (2.8)	16.8 (2.8)
Years of follow-up since questionnaire completion				
Mean (±SD)	11.2 (1.4)	11.2 (1.5)	11.2 (1.3)	11.1 (1.6)

A Women who gave birth to both a singleton and multiples were classified as multiple.
B In case of missing data, median values were assigned (n=509).
C If data on gestational age were missing and the child was alive at birth and had one of the following characteristics: breastfed, a birth weight higher than 1000 grams or a known delivery date, the median gestational age of 37 weeks was assigned (n=315). For each woman with an unknown delivery date (n=95) we imputed the date of first IVF treatment and added 2 years, because 80% of the parous women gave birth within 2 years after IVF treatment.

Breast cancer risk after multiple birth in IVF-treated women

Of the 12,589 IVF-treated women, 317 (2·5%) were diagnosed with breast cancer after their first IVF treatment. Median age at diagnosis was 46·1 years. TABLE II shows the HRs for breast cancer in IVF-treated women according to birth history. Whereas nulliparous women had a significantly decreased breast cancer risk (adjusted HR 0·65, 95% CI (0·48-0·88)), mothers of multiples had a 1·44-fold significantly increased risk of breast cancer (95% CI (1·06-1·97)) compared with women who had singleton pregnancies only. HRs were similar when follow-up started at the date of questionnaire completion (0·63 [95% CI (0·45-0·88)] for nulliparous women and 1·59 [95% CI (1·15-2·19)] for mothers of multiples). Breast cancer risk was more strongly increased in women with higher-order multiples or two multiples (P trend <0·001).

FIGURE 1A presents the cumulative incidence of breast cancer associated with birth history. From age 37 onwards the cumulative incidence of breast cancer among mothers of multiples was higher than among mothers of singletons and nulliparous women (cumulative incidences at age 45 of 2·3%, 1·2% and 1·1 %, respectively). FIGURE 1B shows that women with a history of higher-order multiples or more than one multiple birth had the highest cumulative incidence of breast cancer (3·3 % for a 45-year-old woman).

Breast cancer risk and embryo implantation potential

In subgroup analyses restricted to pregnancies conceived after IVF (n=5,256, 68·1%) results were comparable to those from analyses including all pregnancies (HR of 0·65 (95% CI (0·48-0·89)) for nulliparous women, and 1·40 (95% CI (1·00-1·97)) for mothers of multiples). For 3,149 women (59·9%) with an IVF pregnancy, the number of transferred embryos could be abstracted from the medical records and linked to the subsequent pregnancy. The majority of women (95·4%) had multiple embryos transferred; 33·9% had a double embryo transfer, 45·0% had three embryos transferred, and 16·5% four or more embryos. TABLE III shows the HRs for breast cancer for women with a known number of transferred embryos; 2,154 had a singleton birth, 580 had a multiple birth that developed from part of the transferred embryos, and 415 women had a multiple birth that developed from all transferred embryos. Compared to women with a singleton birth, women who gave birth to multiples from all embryos transferred were at significantly increased risk to develop breast cancer (adjusted HR 1·86, 95% CI (1·01-3·43)), whereas women who gave birth to multiples after implantation of part of the embryos transferred were not (adjusted HR 1·31, 95% CI (0·76-2·25)).

TABLE II Hazard ratios for breast cancer in IVF-treated women according to birth history

	Follow-up from first IVF treatment				Follow-up from questionnaire A			
	Person years treatment	No. of breast cancers	Crude HR (95% CI)	Adjusted HR B (95% CI)	Total no.	No. of breast cancers	Crude HR (95% CI)	Adjusted HR C (95% CI)
Nulli-parous	89,636	105	0.80 (0.62-1.02)	0.65 (0.48-0.88)	4,771	78	0.74 (0.56-0.98)	0.63 (0.45-0.88)
Singleton birth	94,747	155	1.00 (ref)	1.00 (ref)	5,934	131	1.00 (ref)	1.00 (ref)
Multiple birth	25,523	57	1.48 (1.09-2.01)	1.44 (1.06-1.97)	1,665	54	1.48 (1.08-2.03)	1.59 (1.15-2.19)
Twins	21,832	45	1.37 (0.98-1.91)	1.33 (0.95-1.86)	1,433	42	1.34 (0.94-1.89)	1.43 (1.01-2.03)
Triplets Qua-druplets Two multiples	3,691	12	2.19 (1.21-3.94)	2.15 (1.19-3.88)	232	12	2.38 (1.32-4.30)	2.62 (1.44-4.74)

A 219 women were excluded from these analyses, because they had developed (breast) cancer before questionnaire completion (n=114) or because they refused future linkage with the NCR (n=105).
B Adjusted for calendar year of IVF treatment (< 1990, 1990-1992, 1992-1994, > 1994), number of IVF cycles (1-2 cycles, 3-4 cycles, ≥ 5 cycles), height (< 164 cm, 164-170 cm, 170-173 cm, ≥ 174 cm) and age at first birth (< 25, 25-30, 30-34, ≥ 35/nulliparous).
C Adjusted for age at questionnaire completion (<35, 35-40, ≥ 40), number of IVF cycles (1-2 cycles, 3-4 cycles, ≥ 5 cycles), height (< 164 cm, 164-170 cm, 170-173 cm, ≥ 174 cm) and age at first birth (< 25, 25-30, 30-34, ≥ 35/nulliparous).

TABLE III Hazard ratios for breast cancer in IVF-treated women according to the potential to successfully implant all embryos transferred

Successful implantation of embryos	Total no.	No. of breast cancers	HR (95% CI)	Adjusted HR B (95% CI)
Singleton birth – from all or part of the embryos transferred A	2,154	52	1.00 (ref)	1.00 (ref)
Multiple birth – from part of the embryos transferred	580	18	1.20 (0.70-2.05)	1.31 (0.76-2.25)
Multiple birth – from all embryos transferred	415	14	1.51 (0.83-2.72)	1.86 (1.01-3.43)

A Because of the small number of single embryo transfers, we were not able to analyze singleton pregnancies that resulted from implantation of all or only part of the transferred embryos separately.
B adjusted for age at first IVF treatment (continuous), number of IVF cycles (1-2 cycles, 3-4 cycles , ≥ 5 cycles), height (< 164 cm, 164-170 cm, 170-173 cm, ≥ 174 cm), parity (0,1,2, ≥ 3) and age at first birth (continuous).

FIGURE 1A **Cumulative incidence of breast cancer in IVF-treated women according to birth history**

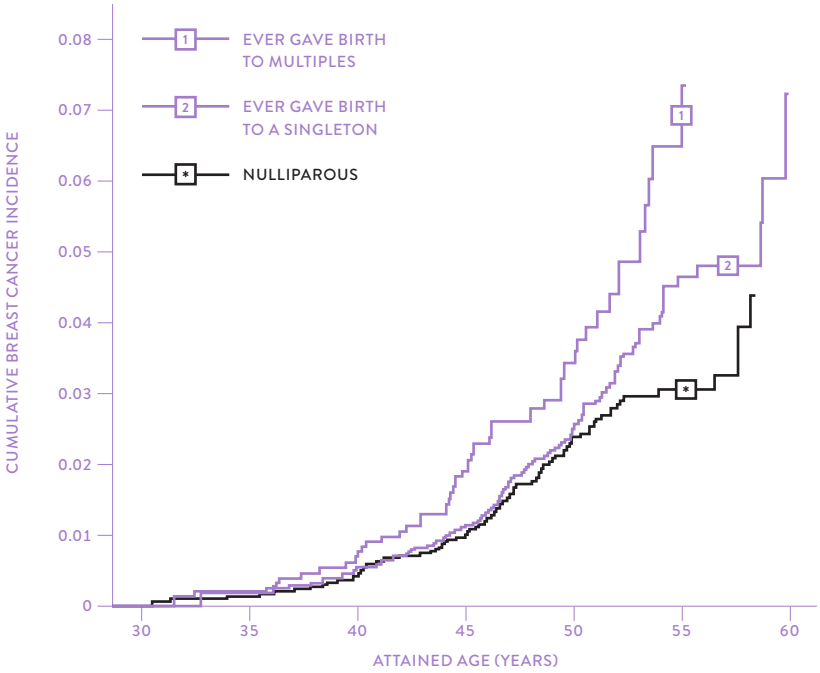
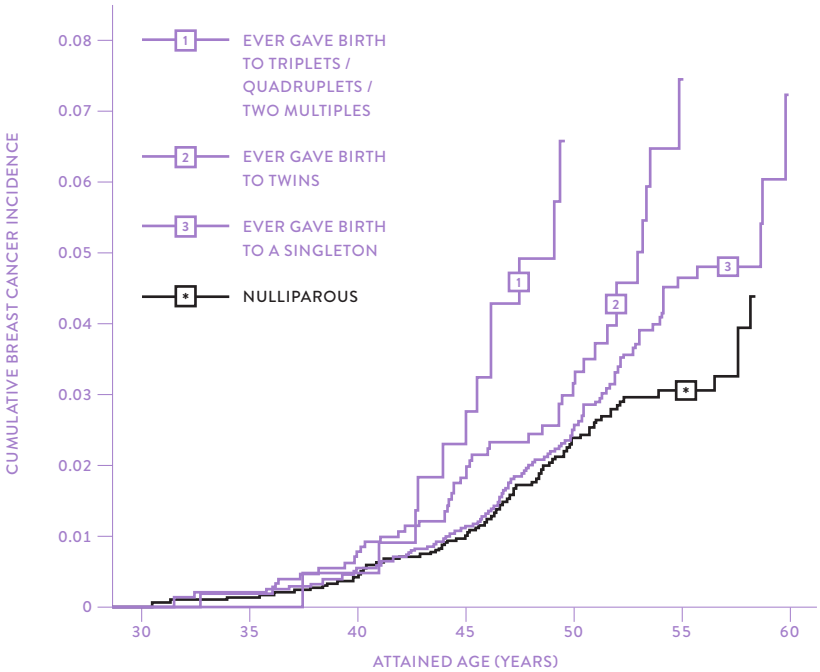


FIGURE 1B **Cumulative incidence of breast cancer in IVF-treated women according to multiple birth history**



DISCUSSION

In this large study of IVF-treated women, mothers of multiples had an 1·44-fold increased risk of breast cancer (95% CI (1·06-1·97)) compared with mothers of singletons. Moreover, women who gave birth to multiples from all embryos transferred had a significantly higher risk (adjusted HR 1·86, 95% CI (1·01-3·43)) than mothers of singletons. This risk was not significantly increased for women with a multiple birth from part of the embryos transferred (HR 1·31, 95% CI (0·76-2·25)). These results support our hypothesis of an association between the potential to successfully implant all embryos transferred and increased breast cancer risk.

Our study is the first study that specifically examined the association between a multiple pregnancy and breast cancer risk in IVF-treated women, so our findings need to be confirmed in other large studies. Recently, another study on breast cancer risk among women seeking subfertility treatment demonstrated a non-significantly decreased breast cancer risk in women who delivered multiples compared with women without a multiple birth (adjusted HR 0·69, 95% CI (0·41-1·16)) [23]. However, this study also included women without IVF-treatment and could therefore not specifically evaluate the effect of a multiple pregnancy conceived by IVF on breast cancer risk. Our study in a homogenous population of IVF-treated women shows a more accurate estimation of breast cancer risk after having multiples by IVF. Furthermore, the number of women exposed to IVF in our study was twice as large, increasing the statistical power to examine the association with a multiple birth.

Our study is also the first to investigate the effects of (in)complete embryo implantation and a multiple birth on breast cancer risk, which can only be examined directly in the IVF setting as number of embryos transferred and subsequent (multiple) pregnancies are known. The association between successful implantation of all embryos transferred and breast cancer risk in our study may be explained by a number of common angiogenic growth factors (e.g. fibroblast growth factor, VEGF) involved in embryo implantation as well as breast cancer progression [14;18;24-25]. Since we recently observed an association between VEGF levels and successful implantation of multiple embryos [13], we hypothesize that elevated levels of VEGF present in women with high potential for multiple implantation may explain the higher breast cancer risk found in these women.

A remarkable finding in our study is the decreased risk of breast cancer in nulliparous women compared with mothers of singletons. In the general population, nulliparity is a well-established risk factor for breast cancer, associated with a 20-40%

higher risk of postmenopausal breast cancer compared with parity before age 25 [26]. However, the nulliparous women in our study are different from nulliparous women in the general population, as all of them intensively attempted to become pregnant through IVF treatment, yet remained nulliparous. Part of these women had a known poor response to ovarian stimulation which has been associated with early menopause [27]. Since early menopause is associated with a decreased risk of breast cancer [28], this might explain the lower breast cancer risk among nulliparous women. However, the OMEGA-study does not have data on menopausal age after questionnaire completion. We therefore performed a sensitivity-analysis with number of oocytes retrieved in the first IVF cycle as a proxy for 'menopausal age', but adjustment for this variable did not materially affect our HRs. In addition, because 80% of the women were diagnosed with breast cancer before the age of 50, it seems unlikely that adjustment for menopausal age would alter our results.

Strengths and limitations

Strengths of our study include the large size of our cohort (including 1,688 IVF-treated women with multiples) and the long-term follow-up (median 16·7 years). We were able to link 96% of our cohort with the NCR and all breast cancers were histologically confirmed. We collected reproductive variables directly from the participating women and for the majority of women (76 %) detailed information on IVF treatments was abstracted from medical records. We also collected data on the number of transferred embryos. However, this was incomplete for part of the women, restricting the power for our subgroup-analysis investigating a possible association between embryo implantation potential, and breast cancer risk. Compared to the other published studies on breast cancer risk among mothers of multiples, our study has certain unique features. First, we included nulliparous women while most studies examined breast cancer risk among parous women only. Second, we included women with higher-order multiple births. Our results indicate that breast cancer risk increases with a history of higher-order multiple births, or more than one multiple birth. Because of the relatively low incidence of higher-order multiples (n=213) and the fact that number of transferred embryos was only available for 62·5 % of the multiple births, we could not reliably investigate whether there is a trend. Finally, by using time-dependent analysis we tried to overcome limitations associated with logistic regression analysis used in previous studies, taking into account a woman's birth history.

A limitation of our analytic approach is that women who had developed breast cancer before questionnaire completion may have been more likely to fill out the questionnaire than women who did not. Although it is unlikely that such selection would be differential according to birth history, this could theoretically lead to overestimation of breast cancer risk when starting observation time at first IVF treatment. However, our sensitivity analyses (in which we eliminated this potential selection bias by starting observation time at questionnaire completion) yielded essentially similar results.

We should also bear in mind that there may be differences in the aetiology of

premenopausal and postmenopausal breast cancer [29]. Since the majority of women were premenopausal when they were diagnosed with breast cancer our results may not apply to postmenopausal breast cancer. More prolonged follow-up of our cohort is needed to examine this issue.

Clinical implications

Although our results were derived from a large nationwide cohort study, these findings should be replicated in other large studies among IVF-treated women. So far, clinical implications are limited. Owing to modern single-embryo transfer strategies, multiple pregnancy rates after artificial reproductive techniques are currently low (around 5%) [30]. In the future, a woman's potential to successfully implant multiple embryos will therefore not be as easily revealed as in our study cohort. Furthermore, the magnitude of the increase in risk for mothers of multiples is comparable with effects of established risk factors (i.e. nulliparity, late age at first birth, early age at menarche) which are currently not used to define risk groups for breast cancer screening. Hence, we do not recommend that IVF-treated women with multiples from all embryos transferred undergo routine screening mammography at an earlier age than recommended for the general population.

Our study results may contribute to novel insights into the pathogenesis of breast cancer. Future studies are needed to examine whether the significant association between a multiple birth from all embryos transferred and breast cancer risk shown in our study is based on common angiogenic growth factors, such as VEGF.

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